International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 9, Issue 9 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.013

Exploring the structural necessities of novel substituted imidazole; inhibitors of nitric oxide synthase with antioxidant properties; in terms of QSAR modeling

Milan* Kumar Maiti[a], Partha Pratim Mahata[a], Pallab Dasgupta[a]
[a]BCDA College of Pharmacy & Technology, 78, Jessore Road (S),
Hridaypur, Kol-700127

Abstract

Nitric oxide (NO) is an oxidative cell signaling molecule to plays a significant role in the prolongation of inflammation, cytokines formation and immunological responses via free radical chain reaction. It helps to regulate turgidity of vessels, insulin secretion, airway tone, and rhythmic contraction and relaxation of GIT, and is involved in angiogenesis and neural development. Different isoform of NOS enzyme have been identified are: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) NOS. To find the structural requirements for more active antioxidant agents, QSAR study was performed on seventeen (17) substituted imidazole by using various statistical algorithm like PCRA, FA-MLR using different software's. QSAR study reveals that increase Wong ford charge at the atom number 8 (C_8) and 12(C_{12}) of common substituted imidazole, may favorable for anti-oxidant activity. The study highlights that the nitric oxide synthase antagonistic activity may increase with the increase of total number of nitrogen atom of whole molecule (nN) as well as Vander wall volume of atoms of any compounds present in this data set and also implies that increase aromatic nitro group or any other electron withdrawing group may be unfavorable for the antioxidant activity. The study also emphasizes that the nitric oxide synthase antagonistic activity may decrease with the increase of electro topological state of atoms and atomic mass of any compounds present in data set. Increase Wong ford charge at atom number 13 'C_{13}' implies that increaser the values of these descriptors are detrimental for biological activity. The average valance connectivity index Chi-3 (X3Av) implies that increase number of non-Hydrogen neighbors and the number of bonds i.e., the sum of all bonds present in the Hydrogen-depleted molecule decrease antioxidant activity.

Keywords: Nitric oxide, Free Radical, Antioxidant, QSAR, Imidazole, PCRA, and FA-MLR

1. Introduction

The gas nitric oxide (NO) acts as stimulator in cellular site and is responsible for pathophysiological change for its free radical like nature [1-3]. It is produce by the enzyme nitric oxide synthases (NOS) from amino acid L-arginine (L-Arg) in aerobic condition [4-6]. The enzyme Nitric oxide synthases (NOSs) are triggering the formation of nitric oxide (NO) from amino acid L-arginine [7]. NO is an important cell signaling bioactive compounds. It helps to regulate turgidity of vessels, insulin secretion, airway tone, and rhythmic contraction and relaxation of GIT, and is involved in angiogenesis and neural development. Different isoform of NOS enzyme have been identified are: neuronal (nNOS), inducible (iNOS), and endothelial (eNOS) NOS. All three isoforms are unique in term of their N-terminal catalytic oxygenase domain, central linker region and C-terminal reductase domain. The NO produce by nNOS is an imperative neurotransmitter for brain growth, memory, learning, and have an efficient cognitive effect. NO produced by eNOS is responsible for regulations osmotic load, blood pressure, and affect blood coagulation by preventing platelet aggregation [8-12]. Beside these, NO have effect on gastric gland, genital and urinary tract by its non adrenergic and non cholinergic effect on nerve endings. The NO derived from iNOS has remarkable cytotoxic effect on the normal immune or inflammatory cells [13-14]. It was obvious that; a group of substituted imidazole nucleous containing molecule acts as inhibitors of both nNOS and eNOS enzymes. As per the previous studies, it was identified that the presence of substituted pyrazole improved nNOS and eNOS inhibitory activity, therapeutic benefit in arthritis and impart antioxidant properties [15-17]. Some of them displayed good capacity of free radical scavenging activity and ability to reduce lipid peroxidation [18]. The present study highlights quantitative structure activity relationship (QSAR) study on the recently synthesized substituted imidazole ring containing molecules.

1.1. Nature of Cytotoxic free radicals

Free radicals are the reactive oxygen and nitrogen atomic species generate by external factors in association with the formation of energy. Free radicals are an atom or group of atoms containing at least one unstable unpaired electron with unstable electronic configuration [3, 18]. Free radicals are highly reactive species and have an intention to set a chain reaction for own stability by producing diriment neutral bioactive species. Free radicals are generated within tissue fluid under stress and also in normal body homeostasis processes are under control and less lethal but have more detrimental effect if it is produce in large excess for longer duration of time [19]. It was evident that a small number of free radicals are essential for our normal body functions but this should up to certain extent, but present day's lifestyle status can lead to generate excess free radicals [20].

1.2. Formation of Free Radicals

Oxygen is required for the generation of all ROS, RNS, and reactive chlorine species. The major reactions for the production of oxygen and nitrogen free radicals in the body are illustrated in Fig. 1. The different isoforms of NO: nNOS (originate in nerve; also known as NOS-I), iNOS (found in activated macrophages and liver; also known as NOS-II), and eNOS (originate in vascular endothelial cells; also known as NOS-III) [10-11]. All NOS isoforms require oxygen, tetrahydrobiopterin, nicotinamide adenine dinucleotide phosphate (NADPH), calmodulin, flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and heme for catalytic activity, whereas Ca^{2+} is essential for nNOS and eNOS activity [1, 3]. In contrast, superoxide is generated from O2 by multiple pathways:

1. Activation of the glutamatergic transmitter system which increases the intracellular calcium ion concentration and induces the formation of NADPH oxidation by NADPH oxidase [1, 3].

2. Oxidation of xanthine or hypoxanthine by xanthine oxidase is involve to generate superoxide radicals [1,3].
3. Oxidation of reducing equivalents (e.g., nicotinamide adenine dinucleotide [reduced; NADH], NADPH, and FADH2 [FAD reduced]) via the mitochondrial electron transport system indirectly involve in generation of free radicals [1, 3].
4. Increase hydrogen peroxide formation with in tissue fluid by free radical and super oxide dismutase [1, 3].
5. Production of peroxynitrile ($ONOO^-$) from NO and superoxide radical, which further produces OH^{*-} free radical [1,3].
6. Hydrogen peroxide is broken down into water and oxygen by the enzyme catalase that responsible for tissue necrosis [1, 3, 21].
7. Autoxidation of monamines (eg: dopamine, epinephrine, and norepinephrine), flavins, and hemoglobin in the presence of trace amounts of transition metals [22].

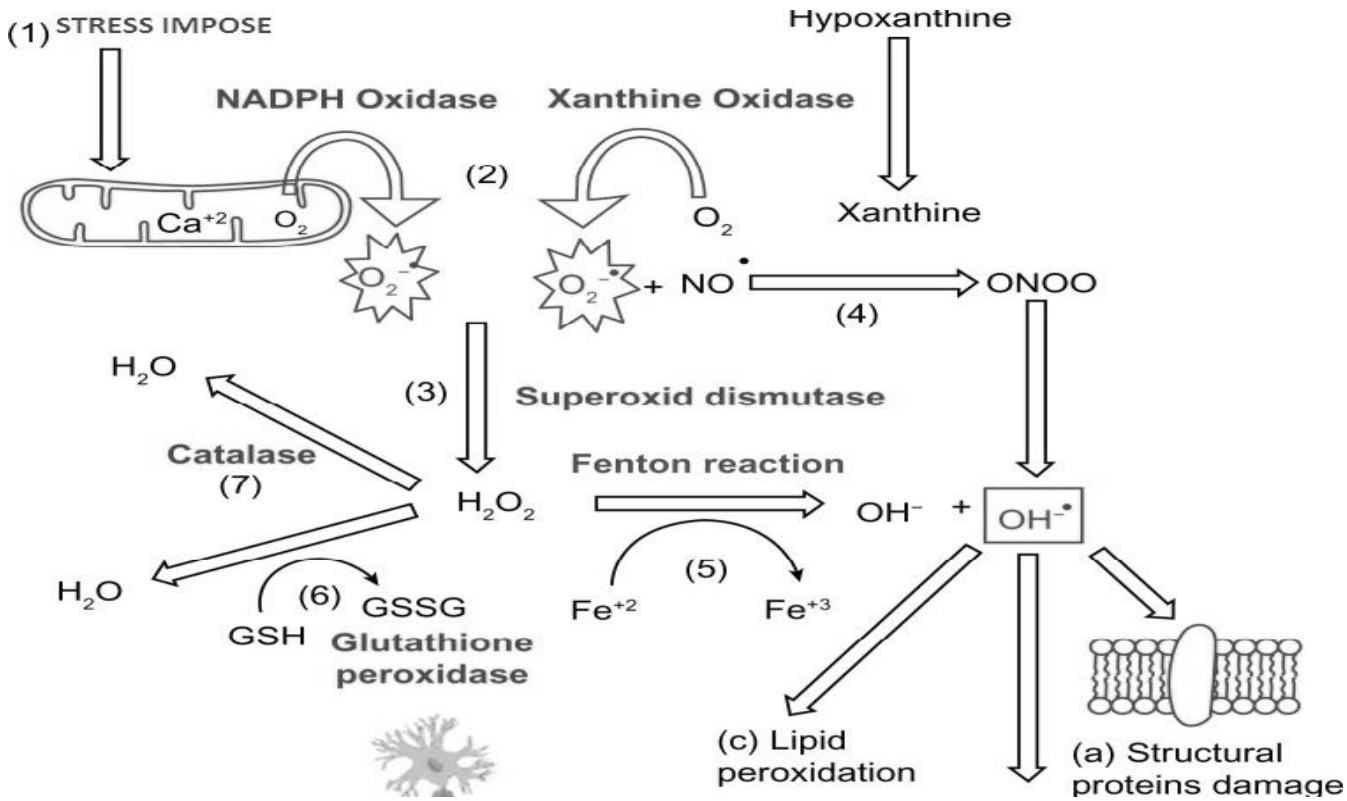


Figure 1. Generation and effects of free radicals in tissue fluid

Peroxidation reactions with the production of lipid peroxyl and alkoxyl radicals, collectively called *chain propagation*, occur in mammalian cells, such that oxygen free radicals may cause damage far in excess of their initial reaction products [23]. The mitochondrial electron transport system is a source of superoxide. Because NADH, NADPH, and FADH2 are produced almost exclusively via the aerobic metabolism of protein, fat, and glucose, an increase in dietary energy intake enhances mitochondrial free radical production, which results in oxidative stress [24]. Thus, calorie restriction reduces the generation of free radical species and retards aging in animals. Under physiologic conditions, approximately 2 % of the O2 consumed by the body is converted into superoxide and other ROS [25]. Throughout the life cycle, any person may be at a risk of oxidative stress induced by high rates of oxygen use, the autoimmune activation of immune system cells, and environmental factors [26].

Prolonged exposure to free radicals, even at a low concentration, may result in the damage of biologically important molecules and potentially lead to DNA mutation, tissue injury, and disease [19]. Thus, although molecular oxygen is absolutely essential for aerobic life, it can be toxic under certain conditions. This phenomenon has been termed the *oxygen paradox* [27].

1.3. Biological Roles of NO as Free Radicals

Free radicals may play an important role in the origin of life and biological evolution, implicating their beneficial effects such as signal transduction, gene transcription, and regulation on the organisms [19]. Also, physiologic levels of NO produced by endothelial cells are essential for regulating the relaxation and proliferation of vascular smooth muscle cells, leukocyte adhesion, platelet aggregation, angiogenesis, thrombosis, vascular tone, and haemodynamics [28]. In addition, NO produced by neurons serves as a neurotransmitter, and NO generated by activated macrophages is an important mediator of the immune response. The radiation-induced ROS markedly alter the physical, chemical, and immunologic properties of SOD, which further exacerbates oxidative damage in cells [29]. The cytotoxic effect of free radicals is deleterious to mammalian cells and mediates the pathogenesis of many chronic diseases, but is responsible for the killing of pathogens by activated macrophages and other phagocytes in the immune system. Thus, there are “two faces” of free radicals in biology in that they serve as signaling and regulatory molecules at physiologic levels but as highly deleterious and cytotoxic oxidants at pathologic levels, viz. lipid peroxidation, protein carbonylation, and DNA damage explain in figure-2. The thiobarbituric acid reactive substances (TBARS), 8-Oxo-2'-deoxyguanosine (8-oxo-Dg) are the oxidized products of lipid and DNA respectively [26].

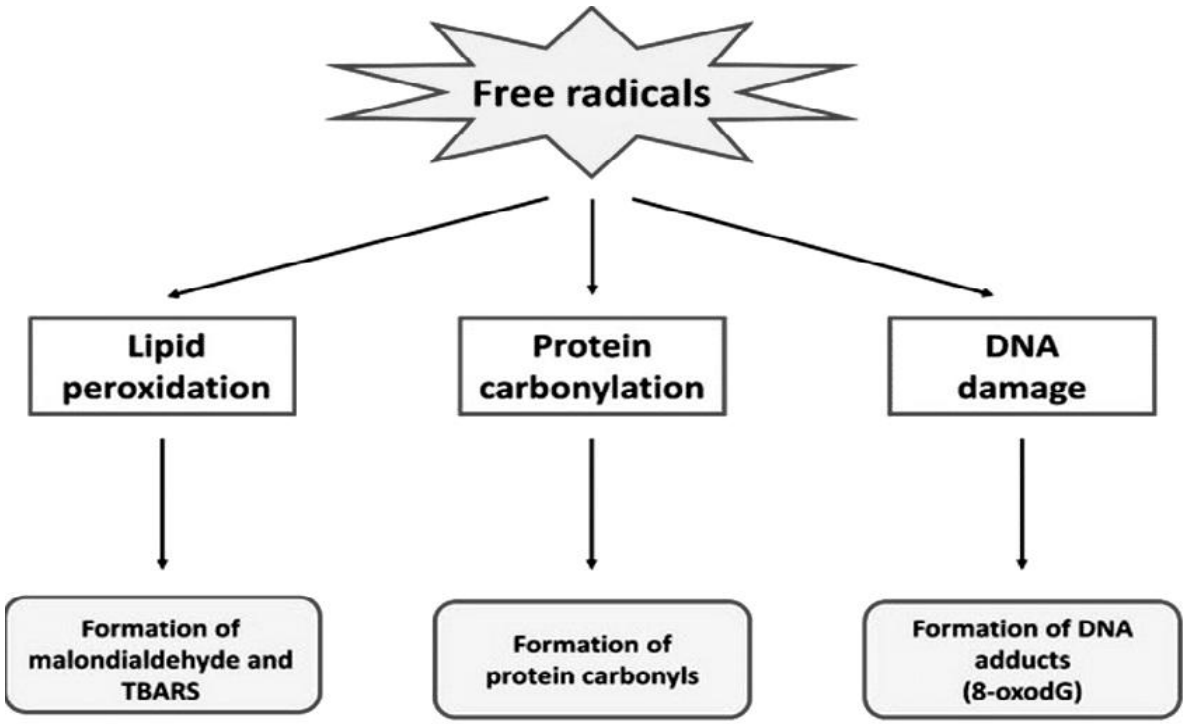


Figure 2. Effects of free radicals on biomolecules

Nitric oxide is a powerful dilator substance that is produced by enzymes located in and around skin blood vessels (nitric oxide synthase enzymes). Clinical studies demonstrate that nitric oxide is intimately involved in many inflammatory skin disorders including rosacea, psoriasis, atopic dermatitis, irritant dermatitis, and allergic dermatitis [30]. In the facial skin, nitric oxide is produced from: a. the inner wall of blood vessels b. Epidermal and dermal cells c. Dilator nerves around blood vessels [31].

After nitric oxide is produced from these cells → it diffuses to nearby blood vessels and binds to receptors on vascular smooth muscle cells → causing potent dilation and substantial increases in blood flow. Topical medications that block nitric oxide production hold the potential to reduce rosacea redness and inhibit many forms of facial flushing [30, 31].

2. QSAR study

To find the structural requirements for more active antioxidant agents, QSAR study was performed on seventeen substituted imidazole as a part of a composite program of rational drug design, discovery and development [32-40]. The general structure of these compounds with arbitrary numbering is shown in **Figure 3**.

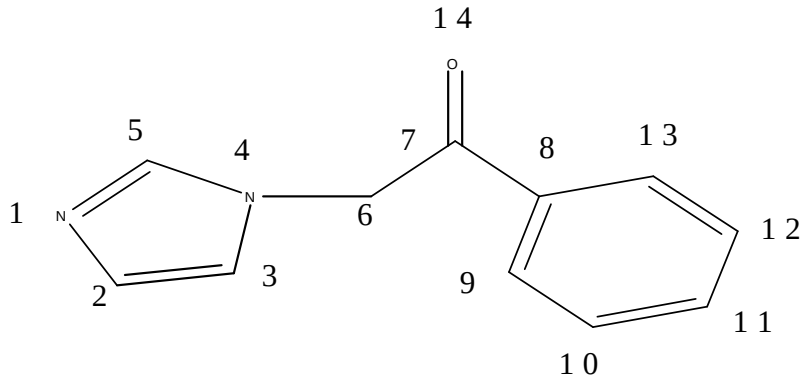


Figure 3. General structure of substituted imidazole, derivatives with arbitrary numbering

2.1. Material Methods

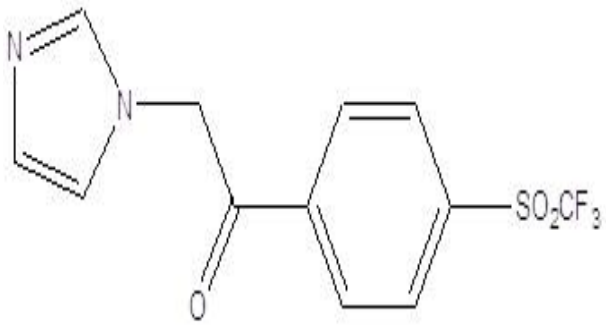
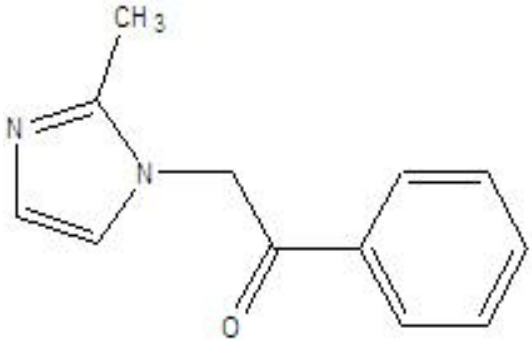
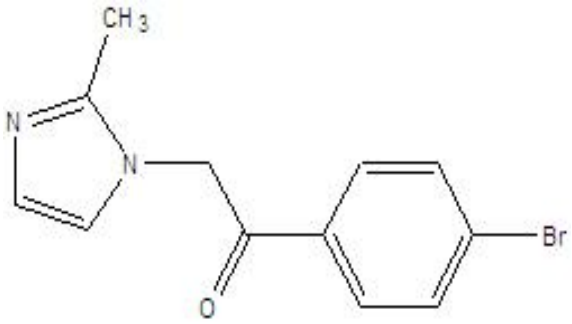
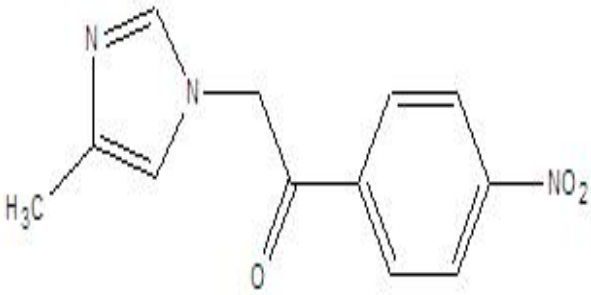
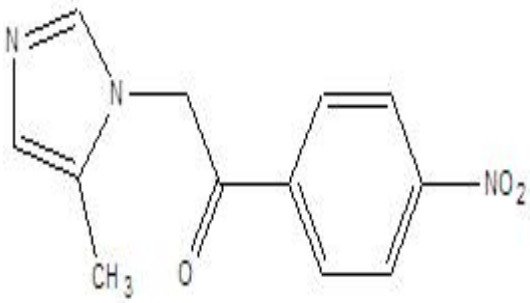
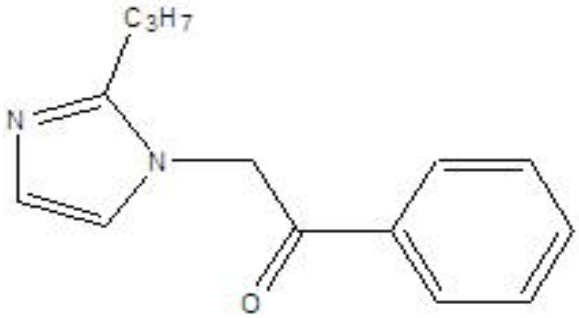
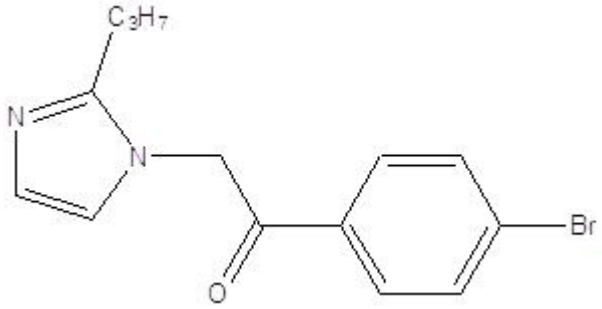
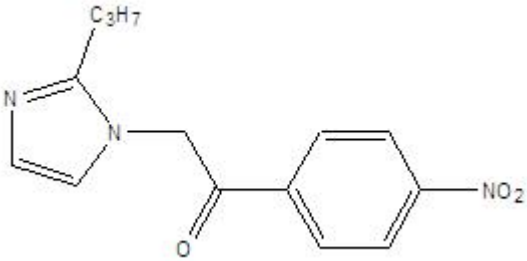
QSAR study was performed on seventeen substituted imidazole, pirazole & triazole derivatives that were synthesized and biologically evaluated previously by Salerno et al. [41]. The functional activities were determined by inhibition of neuronal nitric oxide synthase (nNOS). These activities were expressed as pIC_{50}, and considered as the biological activity parameters for the QSAR study. Here, the negative logarithm of inhibitory activity of these compounds (pIC_{50}) ware used to develop the QSAR models to obtain a linear relationship. The pIC_{50} values and structures of all these compounds are shown in Table 1 and Table 2.

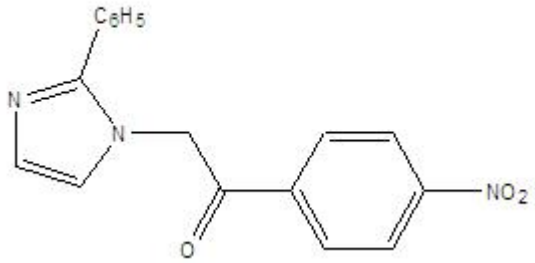
Table 1. Inhibition of neuronal nitric oxide synthase (nNOS) of substituted imidazole derivatives

Cpd[a]	pIC_{50} (M)	Cpd[a]	pIC_{50} (M)	Cpd[a]	pIC_{50} (M)
CPD1	3.346787	**CPD7**	3.346787	**CPD13**	4.440692
CPD2	3.301030	**CPD8**	4.397940	**CPD14**	4.397940
CPD3	3.207608	**CPD9**	4.397940	**CPD15**	4.200659
CPD4	4.070581	**CPD10**	4.346787	**CPD16**	4.187087
CPD5	4.045757	**CPD11**	3.920819	**CPD17**	4.323307
CPD6	3.124939	**CPD12**	4.124939		

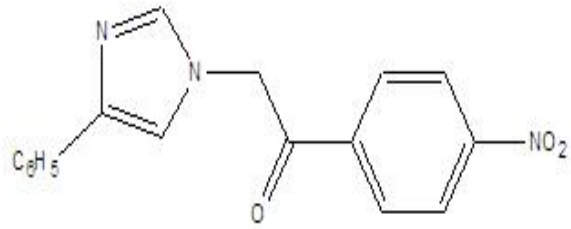
[a]Compound number;

Table 2. Structure of seventeen substituted imidazole derivatives

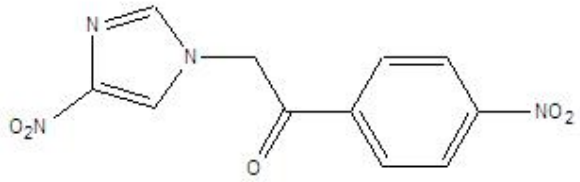
 **CPD1**	 **CPD2**
 **CPD3**	 **CPD4**
 **CPD5**	 **CPD6**
 **CPD7**	 **CPD8**



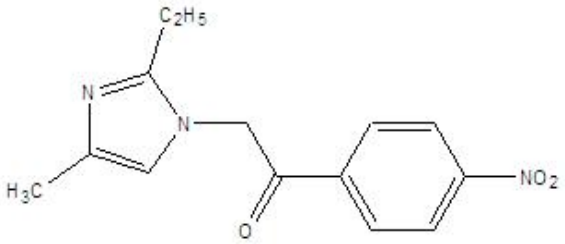
CPD9



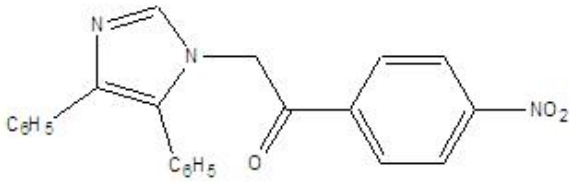
CPD10



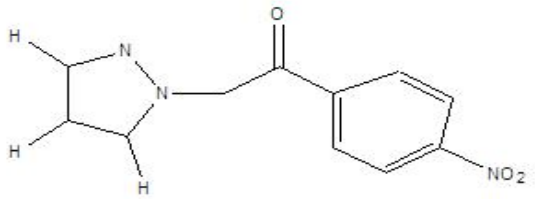
CPD11



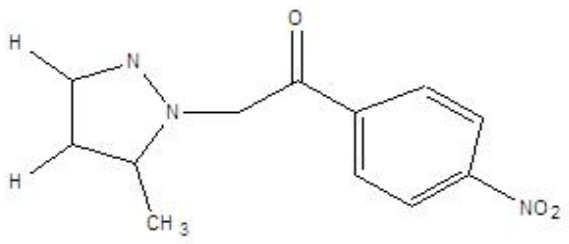
CPD12



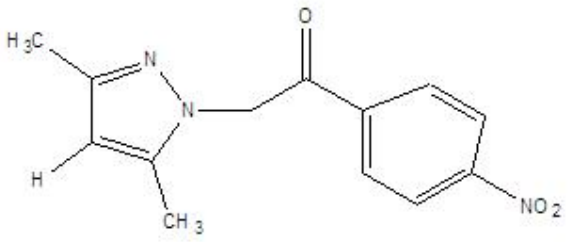
CPD13



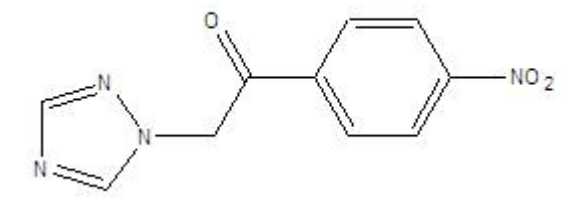
CPD14



CPD15

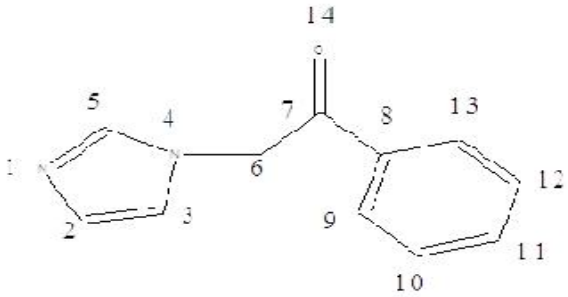


CPD16



CPD17

Gen.tructure



QSAR study was performed using electronic descriptors like atomic charges (Q), electrostatic potential charges (EP), constitutional descriptors as well as all dragon descriptors were used to consider quantitatively the effect of the structural variation on $pMIC_{50}$. Apart from these, *indicator parameters* were also calculated but not used to highlight a structural feature present in some of these molecules in the dataset that confers unusual activity or lack of it to these particular members. Different statistical methods were used for the development of QSAR models are correlation analysis [50], factor analysis (FA) [51,52], multiple linear regression analysis (MLR) [46], Principal component regression analysis (PCRA) [48-49], as well as factor analysis multiple linear regression analysis ((FA-MLR) [47]. The statistical qualities of the models were justified by parameters like correlation coefficient (R), adjusted R^2 (R^2_A), variance ratio (F) at specified degrees of freedom, probability factor related to F-ratio (p) and standard error of estimate (SEE). Leave-one-out (LOO) cross validation method [48] was applied to validate QSAR models.

The descriptors that were used as independent variables include different informational content indices, electronic descriptors like Wang-Ford charges, frontier electron density, and constitutional descriptors. Moreover different atom based and whole molecular quantum chemical descriptors were also used. Prior to calculation, atoms of these molecules were numbered arbitrarily keeping the serial number of atoms identical for all molecules.

Different descriptors were calculated by using different type software's in order to develop QSAR models. Different atom based and whole molecular quantum chemical descriptors were calculated by using *Chem. 3D Pro package*. The *2D* structure of all molecules was drawn separately in *Chem draw ultra* ver 5.0 [42]. To create the 3-D models, all these structures were transformed to *Chem 3D* ver 5.0. Energy minimization of individual structure was done under MOPAC module using RHF (restricted Hartee-Fock: closed shell) wave function [43]. These energy minimized geometry were subjected to calculate Wang-Ford charges. Calculation of different quantum chemical descriptors were done using computer program *Hyperchem Release 7.0 Pro. Package* [44]. *Dragon* software [45] was utilized for the calculation of different topological, geometrical and constitutional descriptors.

3. Results

3.1. Correlation Analysis [50]

Correlation analysis [50] was carried out with the response variable and independent parameters of the training set. Intercorrelated independent parameters were not considered for multiple linear regression analysis and eliminated stepwise depending on their individual correlation with the biological activity. The calculated values of selected independent parameters to develop QSAR models are listed in **Table 3.**

Table 3. Calculated values of selected independent parameters

CPD	pIC50	C_8	C_{12}	C_{13}	EP_9	nN	X_3AV	JGI_4	H7m
1	3.347	-0.133	-0.041	-0.042	-0.247	2.000	0.104	0.051	0.072
2	3.301	-0.224	-0.137	-0.017	-0.185	2.000	0.090	0.033	0.002
3	3.208	-0.171	0.048	-0.071	-0.355	2.000	0.105	0.044	0.003
4	4.071	-0.110	-0.044	-0.103	-0.117	3.000	0.080	0.041	0.100
5	4.046	-0.120	-0.044	-0.101	-0.118	3.000	0.082	0.043	0.083
6	3.125	-0.270	-0.152	0.015	-0.159	2.000	0.093	0.037	0.006
7	3.347	-0.204	0.044	-0.055	-0.321	2.000	0.106	0.046	0.006
8	4.398	-0.016	-0.012	-0.169	-0.124	3.000	0.085	0.045	0.072
9	4.398	-0.170	-0.050	-0.041	-0.117	3.000	0.079	0.039	0.061
10	4.347	-0.181	-0.030	-0.047	-0.132	3.000	0.080	0.041	0.127
11	3.921	-0.173	-0.066	-0.024	-0.101	4.000	0.072	0.054	0.275
12	4.125	-0.128	-0.049	-0.105	-0.090	3.000	0.084	0.052	0.104
13	4.441	-0.140	-0.039	-0.045	0.028	3.000	0.079	0.051	0.110
14	4.398	-0.072	-0.036	-0.094	-0.063	3.000	0.092	0.041	0.010
15	4.201	-0.055	-0.038	-0.098	-0.013	3.000	0.098	0.043	0.012
16	4.187	-0.161	-0.070	-0.038	-0.056	3.000	0.082	0.043	0.031
17	4.323	-0.193	-0.057	-0.034	-0.050	4.000	0.075	0.041	0.009

The correlation matrix among the response variable and those selected descriptors is shown in Table 4.

Table 4. Correlation Analysis of variables used to develop QSAR models

	BA	C8	C12	C13	EP9	nN	X4Av	JGI4	H7m
BA	1.00	0.45	0.10	-0.45	0.39	0.47	-0.48	0.13	0.29
C8		1.00	0.39	-0.49	0.35	0.30	0.10	0.30	0.10
C12			1.00	-0.42	-0.39	-0.04	0.27	0.41	-0.02
C13				1.00	-0.11	-0.17	-0.11	-0.20	-0.02
EP9					1.00	0.41	-0.36	0.06	0.25
nN						1.00	-0.47	0.27	0.34
X4Av							1.00	-0.06	-0.18
JGI4								1.00	0.42
H7m									1.00

3.2. Validation of QSAR models

Leave-one-out (LOO) cross validation method was applied to validate the QSAR models developed. Predictive powers of these equations were justified by this method. Predicted residual sum of square (PRESS), total sum of squares (SSY), cross-validated R^2 (R^2_{CV}), standard deviation of error of prediction (SDEP) and standard error of PRESS (S_{PRESS}) were considered for validation of these models.

3.3. Stepwise Regression [46]

Attempts were made to develop QSAR models for the antioxidant activity of substituted imidazole derivatives. By forward stepwise selection method on the basis of F value (F=1.0 for inclusion; F=0.2 for exclusion), Eq. 1 was derived.

pIC_{50} = 10.235 (±0.999) + 0.545 (±1.406) nN – 36.934 (±5.340) G2v – 30.816 (±6.211) JGI4 + 1.754(±0.565) C_8 Eq. (1)

n= 17; R= 0.975; R^2= 0.951; R^2_A = 0.935; F (4, 12) =59.216; p<0.00000; S.E.E. = 0.121; SSY= 3.694; PRESS= 0.475; R^2_{cv}= 0.871; SDEP= 0.028; S_{PRESS}= 0.036.

Where n is the number of data points i.e., the number of substituted imidazole present in the data sets. The 95% confidence intervals of the regression coefficient are shown in parentheses. Eq. (1) explains 95.20% and predicts 93.50 % variances of the antioxidant activity. Values within the parenthesis are the standard error of corresponding parameters. Eq. 1 suggests importance's of the Wong ford charge at the atom number 8 (C_8), number of nitrogen atom of whole molecule (nN), 2nd component symmetry directional WHIM descriptor / weighted by atomic Vander wall volume (G2v), JGI4 is the mean topological charge index of order 4 of whole molecules in the biological activity. The positive coefficients of those the above mentioned parameters imply that higher values of these parameters may correspond to the higher binding affinity towards the free radical or oxidant as far as the better antioxidant activity is concerned. Electrophilic attacks may likely to occur where the coefficients of Wong ford charge are negative. Eq. 1 also suggests that electrophilic substitutions are not favorable at C_8 (Figure 1) i.e., the higher negative electrostatic potential at C_8 be unfavorable to the nitric oxide synthase antagonistic activity. The G2s is the 2nd component symmetry directional WHIM index weighed by atomic electrotopological state.

The positive coefficient of *G2v* emphasizes that the nitric oxide synthase antagonistic activity may increase with the increase of Vander wall volume of atoms of any compounds present in this data set. From this various topology associated matrix concept, the topological charge index (GG_k) and mean topological charge index (JG_k) are appear as descriptor in QSAR and QSPR study.

The *Topological Charge Index* 'GG_k' and *Mean Topological Charge Index* [53, 54] 'JG_k' is defined as:

$$GG_k = ½ \sum \sum | CT_{ij} | \delta (k.d_{ij})$$
$$JG_k = GG_k / n\text{-}1$$

Where, n is the total number of non-hydrogen atom in the molecule, δ (k, d_{ij}) is Kronecker delta. Numerically δ (k, d_{ij}) is 1 if d_{ij} is equal to k and otherwise it is 0 (zero). The positive coefficient associated with the mean topological charge index of order 4 (JGI4) suggests that it may be advantageous for the antagonistic activity [53,54].

The Observed (Obs) vs. LOO-predicted (Pred) activities of Eq. 1 is shown in Figure 4.

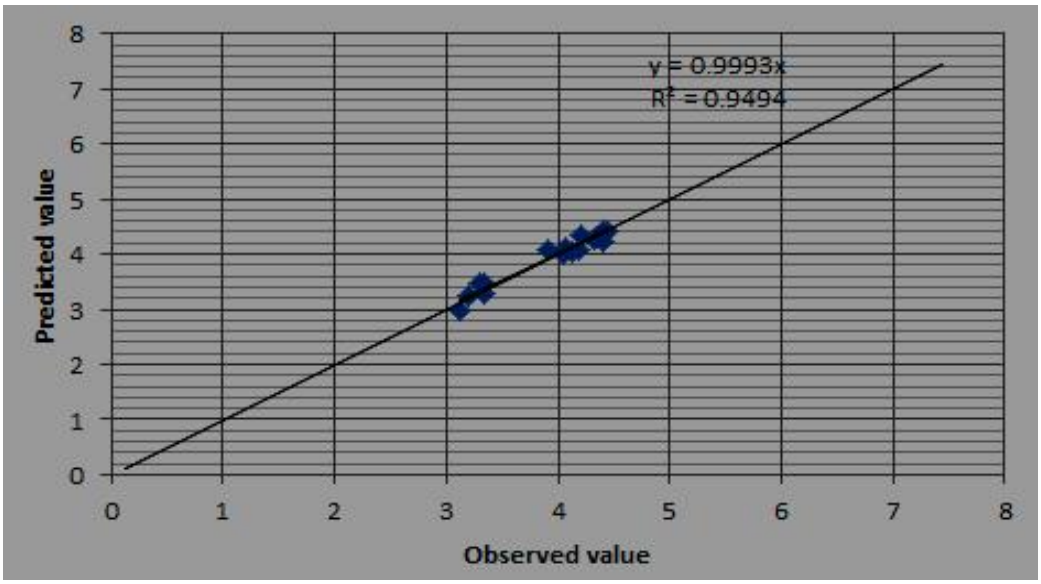


Fig. 4: Observed (Obs) vs. LOO-predicted (Pred) activities of Eq. 1

Attempts were made to develop another QSAR models for the antioxidant activity of substituted imidazole derivatives for whole data set. By forward stepwise selection method on the basis of F value (F=1.2for inclusion; F=0.1 for exclusion), Eq. 2 was derived.

pIC_{50} = 9.018 (±0.832) – 0.970 (±0.079) nNO_2Ph – 5.500 (±0.696) H_7m – 29.054 (±4.246) *Gs* – 1.606 (±0.714) C_{13} Eq. (2)

n= 17; R= 0.977; R^2= 0.955; R^2_A =0.940; F (4, 12) =64.165; p<0.0000; S.E.E. = 0.117; SSY= 3.694; PRESS= 0.718; R^2_{cv}= 0.805; SDEP= 0.042; S_{PRESS}= 0.055.

Where n is the number of data points i.e., the number of substituted imidazole, pirazole & triazole moiety containing compounds present in the data sets. The Eq. (2) explains 97.70% and predicts 95.50% variances of the inhibition higher binding affinity towards the free radical or oxidant as far as the better antioxidant activity is concerned. In Eq. (2) negative coefficients of number of aromatic nitro group, the dragon functional descriptor (nNO2Ph) is a sign of that with the increase of nNO_2Ph decrease antagonistic activity. The Eq. (2) also implies that increase aromatic nitro group or any other electron withdrawing group may be unfavorable for the antioxidant activity. Importance's of other descriptors towards the nitric oxide synthase antagonistic activity be fond of G total symmetry index weighed by atomic electrotopological state 'Gs', Wong ford charge at atom number 13 'C_{13}' *and* H autocorrelation of lag 7 weighted by atomic mass 'H_7m' are also observed. The negative coefficients all of these descriptors are implies that increaser the values of these descriptors are detrimental for biological activity.

The 'Gs' is the G total symmetry index weighed by atomic electrotopological state i.e., a WHIM descriptor [55]. The negative coefficient of 'Gs' and '*H7m*' emphasizes that the nitric oxide synthase antagonistic activity may decrease with the increase of electrotopological state of atoms and atomic mass of any compounds present in this data set. The Observed (Obs) vs. LOO-predicted (Pred) activities of Eq. 2 is shown in Figure 5.

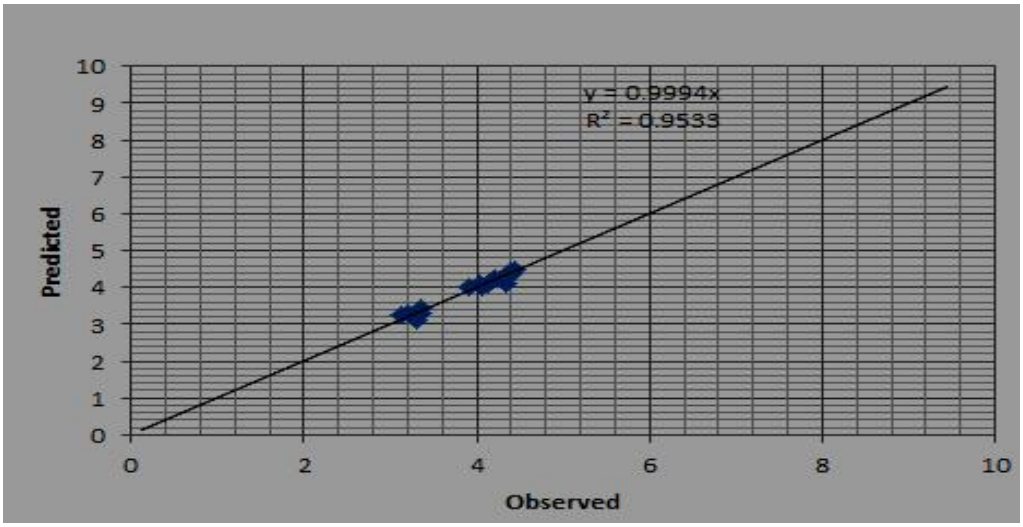


Figure 5: Observed (Obs) vs. LOO-predicted (Pred) activities of Eq. 2

3.4. Factor analysis- Multiple linear regression (FA-MLR) [47]

Factor analysis was performed after VARIMAX rotation and different combinations of parameters having factor loading of more than 0.6(excluding very poorly loaded factors) were subjected to multiple linear regression. Intercorrelation less than 0.5 in between variables were taken in consideration for the development of equations. The results evolved with Eq. (3) with five descriptors after regression analysis:

pIC_{50} = 5.991 (±0.342) – 2.160 (±0.616) EP_9 + 8.133 (±1.115) C_{12} – 0.125 (±0.035) *H0m* –12.102 (±4.291) *X3Av* Eq. (3)

n= 17; R= 0.972; R^2= 0.946; R^2_A =0.928; F (4, 12) =52.345; p<0.0000; S.E.E. = 0.129; SSY= 3.694; PRESS= 0.340; R^2_{cv}= 0.907; SDEP= 0.020; S_{PRESS}= 0.026.

It was observed that 6 factors can explain the data matrix to the extent of 97.20% and predicts 94.60 %. The pIC_{50} is highly loaded with factor 1 and 3, moderately loaded with factor 2 and 6, and poorly loaded with factor 4 and 5. The factor loading of these parameters are shown in Table 3. The newly developed descriptor in that equation is the electro topological charge at atom number 9 (EP_9), Wong ford charge at atom number 12 (C_{12}), H autocorrelation of lag 0 weighted by atomic mass '*H0m*', and average valance connectivity index Chi-3 (X3Av) *are* also observed. The positive coefficient of (C_{12}) emphasizes that the nitric oxide synthase antagonistic activity may increase with the increase charge at atom number 12 of any compounds present in this data set. The negative coefficient of (EP_9) emphasizes that the nitric oxide synthase antagonistic activity may increase with the decrease of electro topological charge at atom number 9 of any compounds present in this data set. The following Kier-Hall Connectivity Indices are calculated from the Hydrogen-depleted molecular graph (Kier & Hall, 1986) [56, 57].

1. Connectivity index Chi-0 through Chi-5
2. Average Connectivity index Chi-0 through Chi-5
3. Valence Connectivity index Chi-0 through Chi-5
4. Average Valence Connectivity index Chi-0 through Chi-5

Connectivity indices Chi-0 through Chi-5 are defined as follows. The Connectivity index Chi-0 is defined as:-

$$\chi^0 = \sum_{i=1}^{n} \delta_i^{-1/2},$$

Where, n is the number of nodes in the Hydrogen-depleted graph, δi is the vertex degree of the i^{th} atom defined as the number of non-Hydrogen neighbors in the molecular graph.

The Average Connectivity index Chi-0 is:

$$\chi^0 A = \chi^0 / n$$

The Connectivity index Chi-1 is defined as:

$$\chi^1 = \sum_{b} (\delta_i \delta_j)^{-1/2},$$

Where, b is the number of bonds, the sum runs through all bond in the Hydrogen-depleted molecule, and for each bond δi δj is the product of the vertex degrees of the end atoms i and j.

The Average Connectivity index Chi-1 is: $\chi^1 A = \chi^1 / b$.

Higher Indices: Connectivity indices Chi-m for 2 ≤ m ≤ 5 is defined as:

$$\chi^m = \sum_{k=1}^{K} (\text{II}\,\delta_i)_k^{-1/2},$$

Where, $(\text{II}\ \delta i)_k$ is the product of the vertex degrees of the atoms that form a connected sub graph with m edges, and K is the total number of such distinct connected sub graphs (the H-depleted molecular graph) each having m edges. For any m, 0 ≤ m ≤ 5, if we replace the vertex degree δi by the valence vertex degree for each atom i in the Connectivity index Chi-m, then we get Valence Connectivity Indices Chi-m (Kier & Hall, 1981; Kier & Hall, 1983)[56,57]. That is, where, $(\text{II}\ \delta i^v)_k$ is the product of the valence vertex degrees of the atoms that form a connected sub graph with m edges, and K is the total number of such distinct connected sub graphs (the H-depleted molecular graph) each having m edges. The average valance connectivity index Chi-3 (X3Av) implies that increase number of non-Hydrogen neighbors and the number of bonds i.e., the sum of all bonds present in the Hydrogen-depleted molecule decrease antioxidant activity.

Table 5. t- and p- values of equations all above developed equations

		t-value	p-value			t-value	p-value
	Intercept	10.2403	0.0000		Intercept	17.5360	0.0000
	Nn	10.6233	0.0000		EP9	3.5053	0.0043
Eq.1	G2v	-6.9171	0.0000	Eq.3	C12	7.2967	0.0000
	JGI4	-4.9613	0.0003		H0m	-3.5331	0.0041
	C8	3.1049	0.0091		X3Av	-2.8201	0.0155
		t-value	p-value			t-value	p-value
	Intercept	10.844	0.000		Intercept	47.7548	0.0000
	nNO2Ph	12.302	0.000		*f3*	-3.0260	0.0097
Eq.2	H7m	-7.900	0.000	Eq.4	*f1*	2.4763	0.0278
	Gs	-6.843	0.000		*f4*	1.8563	0.0862
	C13	-2.250	0.044				

3.5. Principal component regression analysis (PCRA)

In PCRA [48, 49] factor scores were obtained from factor analysis on the data matrix containing all independent variables. Regression analysis was performed considering factor scores as predictor variables. Here, eight factor scores were extracted by principle component method and then rotated by VARIMAX rotation. These factor scores were used as independent parameters for developing QSAR equations. As factor score contains information for the different descriptors, the chance for loss of information is less. Using forward selection technique the following equation was developed:

pIC_{50}= *3.952 (±0.083) – 0.258 (±0.085)* f_3 + *0.211 (±0.085)* f_1 + *0.158 (±0.085)* f_4 Eq (5)

n = 17; R= 0.768; R^2= 0.590; R^2_A= 0.496; F (3, 13) = 6.244; p<0.0001; S.E.E = 0.341; R^2_{cv}= 0.519; SSY = 3.694; PRESS = 1.776; SDEP=0.126; S_{PRESS}= 0.136.

Eq. (7) explains 76.80% and predicts 59 % variances of the biological activity and shows the importance of factor 3, 1and 4. This equation also shows that the factor 3 (f_3) is highly significant for that data set and factor loading is maximum among eight factors.

4. Conclusion

A novel class of substituted imidazole were exhibited a potent binding affinity towards the free radical or scavenger of free radical as far as the better antioxidant activity is concerned. These compounds may significantly reduce free radical induce disorder like cancer, neuro-degenerative disorder and were suggested to have potential therapeutic utility as antioxidant for the treatment of lipid peroxidation and cardiovascular disorder.

QSAR study reveals that increase Wong ford charge at the atom number 8 (C_8) and 12(C_{12}), may be cooperative for anti-oxidant activity. Decreasing charges at atom number 8 and 12, and increasing value of the electrostatic potential charges at atom numbers 9 may be detrimental for the nitric oxide synthase antagonistic activity. The study highlights that the nitric oxide synthase antagonistic activity may increase with the increase of total number of nitrogen atom of whole molecule (nN) as well as Vander wall volume of atoms of any compounds present in this data set and also implies that increase aromatic nitro group or any other electron withdrawing group may be unfavorable for the antioxidant activity. The study also emphasizes that the nitric oxide synthase antagonistic activity may decrease with the increase of electro topological state of atoms and atomic mass of any compounds present in data set. Increase Wong ford charge at atom number 13 'C_{13}' implies that increaser the values of these descriptors are detrimental for biological activity. The average valance connectivity index Chi-3 (X3Av) implies that increase number of non-Hydrogen neighbors and the number of bonds i.e., the sum of all bonds present in the Hydrogen-depleted molecule decrease antioxidant activity. The important atoms and substituents of these derivatives for antioxidant activity are shown in Figure 6.

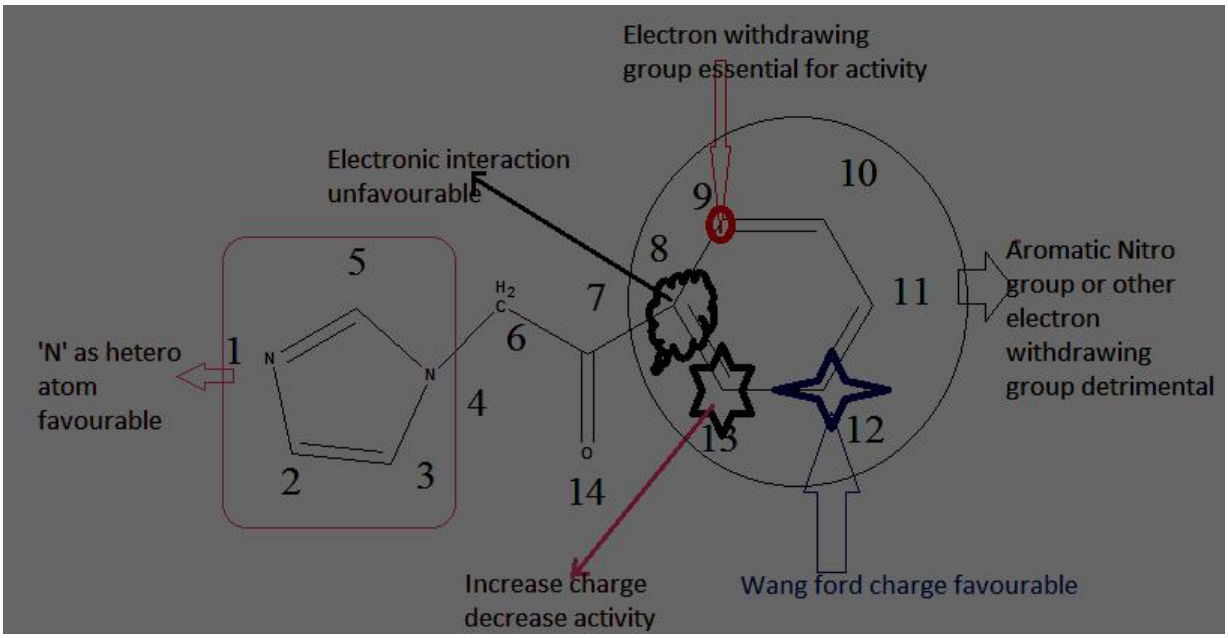


Figure 6. Structural requirements of substituted imidazole derivatives

The result was supported by the energy minimized geometry (shown in Figure 7) obtained during the energy minimizations by AM1 calculations (Compound 5).

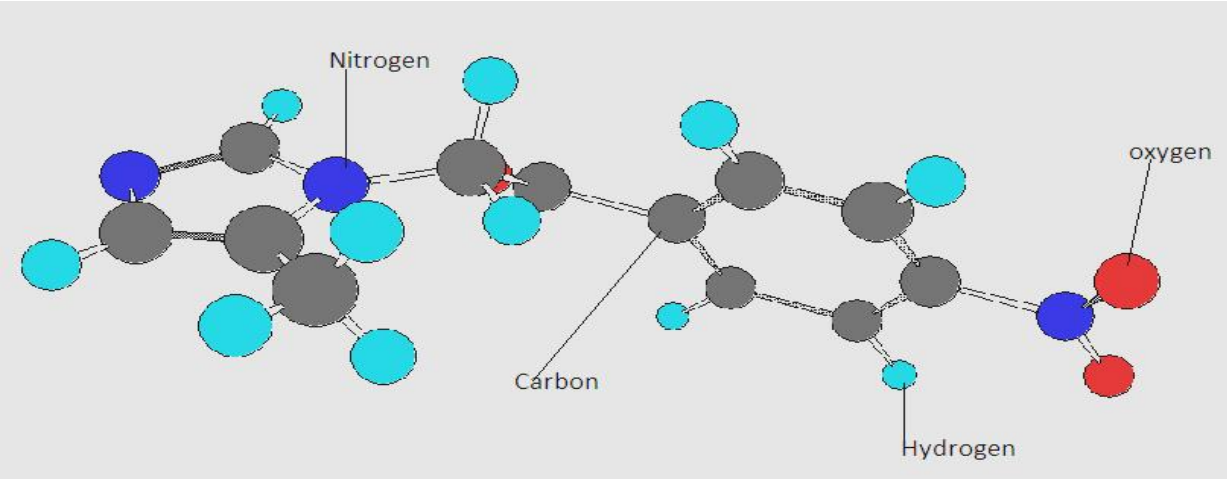


Figure 7. Energy minimized geometry of the most active compound (compound 5)

References

1. Förstermann, U. Sessa, W. C. 2012. Nitric oxide synthases: regulation and function. European heart journal, 33(7), 829–837d. https://doi.org/ 10.1093 /eurheartj /ehr304

2. Hou, Y. C. Janczu, k A. Wang, P G. 1999. "Current trends in the development of nitric oxide donors". Current Pharmaceutical Design. 5 (6): 417–41. PMID 10390607.

3. Phaniendra, A. Jestadi, D. B., & Periyasamy, L. 2015. Free radicals: properties, sources, targets, and their implication in various diseases. Indian journal of clinical biochemistry : IJCB, 30(1), 11–26. https://doi.org/10.1007/s12291-014-0446-0
4. Luiking, Y. C. Ten Have, G. A. Wolfe, R. R. & Deutz, N. E. 2012. Arginine de novo and nitric oxide production in disease states. American journal of physiology. Endocrinology and metabolism, 303(10), E1177–E1189. https://doi.org/10.1152/ajpendo.00284.2012
5. Esplugues, J. V. 2002. NO as a signalling molecule in the nervous system. British journal of pharmacology, 135(5), 1079–1095. https://doi.org/ 10.1038/sj. bjp. 0704569
6. Alderton, W. K. Cooper, C. E. & Knowles, R. G. 2001. Nitric oxide synthases: structure, function and inhibition. The Biochemical journal, 357(Pt 3), 593–615. https://doi.org/10.1042/0264-6021: 3570593
7. Dennis, J. Stuehr, 2004. Enzymes of the L-Arginine to Nitric Oxide Pathway, The Journal of Nutrition, 134 (10), :2748S–2751 S, https://doi.org/ 10.1093/jn/ 134.10.2748S
8. Mungrue, I.N. Husain, M. Stewart, D.J. 2002. "The role of NOS in heart failure: lessons from murine genetic models". Heart Fail Rev. 7 (4): 407–22. doi: 10.1023/a:1020762401408. PMID 12379825. S2CID 26600958.
9. Al-Azemi, M. Refaat, B.; Amer, S. Ola, B. Chapman, N. Ledger, W. 2010. "The expression of inducible nitric oxide synthase in the human fallopian tube during the menstrual cycle and in ectopic pregnancy". Fertil. Steril. 94 (3): 833–40. doi:10.1016/j.fertnstert.2009.04.020. PMID 19482272.
10. Maulik, S.J. Junyi, Z. Aneesh, T.V. Yamuna, K. 2020. "A DNA-based fluorescent probe maps NOS3 activity with subcellular spatial resolution". Nat. Chem. Biol. (16): 660–6. doi:10.1038/s41589-020-0491-3. PMID 32152543.
11. Liu, J. Hughes, T.E. Sessa, W. C. 1997. "The First 35 Amino Acids and Fatty Acylation Sites Determine the Molecular Targeting of Endothelial Nitric Oxide Synthase into the Golgi Region of Cells: A Green Fluorescent Protein Study". J. Cell Biol. 137 (7): 1525–35. doi:10.1083/jcb.137.7.1525. PMC 2137822. PMID 9199168.
12. Gusarov, I. Nudler, E. 2005. "NO-mediated cytoprotection: Instant adaptation to oxidative stress in bacteria". Proc. Natl. Acad. Sci. U.S.A. 102 (39): 13855–60. Bibcode:2005PNAS..10213855G. doi: 10.1073/pnas.0504307102. PMC 1236549. PMID 16172391.
13. Esplugues, J. V. 2002. NO as a signalling molecule in the nervous system. British journal of pharmacology, 135(5), 1079–1095. https://doi.org/10.1038/sj.bjp.0704569
14. Halder, N. & Lal, G. 2021. Cholinergic System and Its Therapeutic Importance in Inflammation and Autoimmunity. Frontiers in immunology, 12, 660342. https://doi.org/10.3389/fimmu.2021.660342
15. Mukherjee, P. Cinelli, M. A. Kang, S. & Silverman, R. B. 2014. Development of nitric oxide synthase inhibitors for neurodegeneration and neuropathic pain. Chemical Society reviews, 43(19), 6814–6838. https://doi.org/10.1039/c3cs60467e
16. Jing, B. S. Liu, Z. C. Bao, S. W. Xin, H. M. Ming, J. Ming, M. L. Wen, J. T. Xin, H. L. 2019. Novel Pyrazolo[4,3-d]pyrimidine as Potent and Orally Active Inducible Nitric Oxide Synthase (iNOS) Dimerization Inhibitor with Efficacy in

Rheumatoid Arthritis Mouse Model. Journal of Medicinal Chemistry , 62 (8, 4013-4031. https://doi.org/10.1021/acs.jmedchem. 9b00039

17. Mukherjee, P. Li, H. Sevrioukova, I. Chreifi, G. Martásek, P. Roman, L. J. Thomas, L. Poulos, T. L. Richard, B. and Silverman, R. B. 2015. Novel 2,4-Disubstituted Pyrimidines as Potent, Selective, and Cell-Permeable Inhibitors of Neuronal Nitric Oxide Synthase. Journal of Medicinal Chemistry , 58 (3), 1067-1088. https: //doi.org/10.1021/ jm501719e
18. Biswas, M. Haldar, P. K. & Ghosh, A. K. 2010. Antioxidant and free-radical-scavenging effects of fruits of Dregea volubilis. Journal of natural science, biology, and medicine, 1(1), 29–34. https://doi.org/10.4103/0976-9668.71670
19. Lobo, V. Patil, A. Phatak, A. & Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews, 4(8), 118–126. https://doi.org/10.4103/0973-7847.70902
20. Rahman, K. 2007. Studies on free radicals, antioxidants, and co-factors. Clinical interventions in aging, 2(2), 219–236.
21. Nandi, A. Yan, L. J. Jana, C. K. & Das, N. 2019. Role of Catalase in Oxidative Stress- and Age-Associated Degenerative Diseases. Oxidative medicine and cellular longevity, 2019, 9613090. https://doi.org/10.1155/2019/9613090
22. Wang, W. Wu, X. Yang, C. S. & Zhang, J. 2021. An Unrecognized Fundamental Relationship between Neurotransmitters: Glutamate Protects against Catecholamine Oxidation. Antioxidants (Basel, Switzerland), 10(10), 1564. https://doi.org/10.3390/antiox10101564
23. Ayala, A. Muñoz, M. F. & Argüelles, S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative medicine and cellular longevity, 2014, 360438. https://doi.org/10.1155/2014/360438
24. Xiao, W. Wang, R. S. Handy, D. E. & Loscalzo, J. 2018. NAD(H) and NADP(H) Redox Couples and Cellular Energy Metabolism. Antioxidants & redox signaling, 28(3), 251–272. https://doi.org/10.1089/ars.2017.7216
25. Zorov, D. B. Juhaszova, M. & Sollott, S. J. 2014. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiological reviews, 94(3), 909–950. https://doi.org/10.1152/physrev.00026.2013
26. Pizzino, G. Irrera, N. Cucinotta, M. Pallio, G. Mannino, F. Arcoraci, V. Squadrito, F. Altavilla, D. & Bitto, A. 2017 Oxidative Stress: Harms and Benefits for Human Health. Oxidative medicine and cellular longevity, 2017, 8416763. https://doi.org/10.1155/2017/8416763
27. Davies, K. J. 2016. The Oxygen Paradox, oxidative stress, and ageing. Archives of biochemistry and biophysics, 595, 28–32. https://doi.org/10.10 16/j.abb.2015. 11. 015
28. Rajendran, P. Rengarajan, T. Thangavel, J. Nishigaki, Y. Sakthisekaran, D. Sethi, G. & Nishigaki, I. 2013. The vascular endothelium and human diseases. International journal of biological sciences, 9(10), 1057–1069. htt ps:// doi.o rg/10.7150/ijbs.7502
29. Kurutas, E. B. 2016. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. Nutrition journal, 15(1), 71.
30. Adler, B. L. & Friedman, A. J. 2015. Nitric oxide therapy for dermatologic disease. Future science OA, 1(1), FSO37. https://doi.org/10.4155/fso.15.37
31. Holowatz, L. A., Thompson-Torgerson, C., & Kenney, W. L. (2010). Aging and the control of human skin blood flow. *Frontiers in bioscience (Landmark edition)*, *15*(2), 718–739.

https://doi.org/10.2741/3642 S. Samanta, K. Srikanth, S. Banerjee, B. Debnath, S. Gayen, T. Jha, Bioorg. Med. Chem. 12 (2004) 1413-1423.

32. B. Debnath, S. Gayen, A. Basu, B. Ghosh, K. Srikanth, T. Jha, Bioorg. Med. Chem. 12 (2004) 6137-6145.
33. Debnath, B. Gayen, S. Basu, A. Srikanth, K. Jha, T. J. Mol. Mod. 10 (2004) 328-334.
34. Debnath, B. Samanta, S. Gayen, S. Ghosh, B. Basu, A. Jha, T. Internet Electron. J. Mol. Des. 4 (2005) 393-412, www.biochempress.com
35. Samanta, S. Debnath, B. Gayen, S. Ghosh, B. Basu, A. Jha, T. Farmaco 10 (2005) 818-825.
36. Debnath, B. Gayen, S. Basu, A. Ghosh, B. Srikanth, K. Jha, T. 2004. QSAR Study on 5–N–Substituted–2–(Substituted Benzenesulphonyl) Glutamines as Antitumor Agents through Synthesis and Biological Evaluation: Part III January 2. Bioorg. Med. Chem.: 12. 6137-6145.
37. Debnath, B. Gayen, S. Basu, A. Srikanth, K. Jha, T. 2004 Quantitative structure-activity relationship study on some benzodiazepine derivatives as anti-Alzheimer agents . J. Mol. Mod.: 10(5-6) . 328-334.
38. Debnath, B. Samanta, S. Gayen, S. Ghosh, B. Basu, A. Jha, T. 2005. QSAR Study on 5-N-Substituted-2-(Substituted Benzenesulphonyl) Glutamines as Antitumor Agents through Synthesis and Biological Evaluation: Part III. Internet Electron. J. Mol. Des. 4 (6) 393-412, www.biochempress.com.
39. Samanta, S. Debnath, B. Gayen, S. Ghosh, B. Basu, A. Jha, T. 2005. QSAR modeling on dopamine D2 receptor binding affinity of 6-methoxy benzamides Farmaco: 60(10). 818-825.
40. Samanta, S. Alam, Sk. M. Panda, P. Jha, T. 2006. Pharmacophore Mapping of Tricyclic Isoxazoles for Their Affinity towards Alpha–2 Adrenoreceptors Internet Electron. J. Mol. 5 (10) 503-514. www.biochempress.com.
41. Samanta, S. Gayen, S. Ghosh, B. Panda, P. Srikanth, K. Jha, T. 2006. QSAR analysis of some indirubin derivatives as potent and selective inhibitors of cyclin–dependent kinases and glycogen synthase kinase–3. Int. J. Appl. Chem. 2 (10) 169-180.
42. Chem 3d Pro version 5.0 and Chem Draw Ultra version 5.0 are programs of Cambridge Soft Corporation, USA
43. Debnath, B. Gayen, S. Samanta, S. Basu, A. Ghosh, B. Jha, T. 2006. QSAR study on some synthesized and biologically evaluated glutamine analogs as possible anticancer agents, Ind. J. Chem. 45 (A) 93-99.
44. Hyper Chem Professional' software – version 7.0 of Hypercube Inc. www.hyper.com
45. DRAGON web version 2.1 is a QSAR software developed by Milano Chemometrics and QSAR Research Group, Dipartimento di Scienze dell' Ambiente e del Territorio Universitadegli Studio di Milano – Bicocca.
46. Halder, A. K. Saha, A. Jha, T. 2013. Exploring QSAR and pharmacophore mapping of structurally diverse selective matrix metalloproteinase-2 inhibitors. The Journal of Pharmacy and Pharmacology, 65(10) :1541-1554
MID: 24028622
47. Halder, A.K. Adhikari, N. Maiti, M.K. Jha, T. 2010. Synthesis, pharmacological activity and comparative QSAR modeling of 1,5-N,N 0-substituted-2-(substituted naphthalenesulphonyl) glutamamides as possible anticancer agents, Eur. J. Med. Chem. 45 . 1760–1771
48. Samanta, S. Debnath, B. Basu, A. Gayen, S. Srikanth, K. Jha, T. 2006. Exploring QSAR on 3-aminopyrazoles as antitumor agents for their inhibitory activity of CDK2/cyclin A. Eur. J. Med. Chem. 41 (10) 1190-1195.

49. Leach, A.R. Molecular modeling principles and applications, second ed., Prentice Hall, 2001.
50. Adhikari, N. Maiti, M. K. Jha, T. Predictive comparative QSAR modelling of (phenylpiperazinyl-alkyl) oxindoles as selective 5-HT1A antagonists by stepwise regression, PCRA, FA-MLR and PLS techniques Eur. J. Med.Chem. 45 (2010) 1119–1127.
51. Adhikari, N. Maiti, M. K. Jha, T. 2010. Exploring structural requirements of 1-N-substituted thiocarbamoyl-3-phenyl- 2-pyrazolines as antiamoebic agents using comparative QSAR modeling, Bioorg. Med. Chem. Letters: 20. 4021–4026.
52. Leonard, J.T. Roy, K. 2006 Biological Activity and Toxicity: A Conceptual DFT Approach Bioorg. Med. Chem. 14 . 1039–1045.
53. Sendecor, G.W. Cochran, W.G. Multiple Regressions in Statistical Methods, Oxford, New Delhi, India.
54. Rios-Santamarina, I. Garcia-Domenech, R. Galvez, J. Cortijo, J. Santamaria, P. & Marcillo, E. 1998. Bioorg. Med. Chem. Lett. 8, 477-482.
55. Galvez, J. Garcia, R. Salabert, M. T. Soler, R. J. 1994, Chem. Inf. Comp. Sci. 34 , 520-525.
56. Kier, L . B. Hall, L. H. 1986. Molecular Connectivity in Structure-Activity Analysis, J. Wiley & Sons, New York.
57. Kier, L. B, Hall, L .H .1986. Molecular Connectivity in Structure-Activity Analysis. RSP-Wiley, Chichetser (UK).

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Pharmaceutical Sciences
DOI:10.22192/ijarbs.2022.09.09.013	

How to cite this article:
Milan Kumar Maiti, Partha Pratim Mahata, Pallab Dasgupta. (2022). Exploring the structural necessities of novel substituted imidazole; inhibitors of nitric oxide synthase with antioxidant properties; in terms of QSAR modeling. Int. J. Adv. Res. Biol. Sci. 9(9): 137-155.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.09.013